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KAIROMONES AND THEIR USE FOR MANAGEMENT OF ENTOMOPHAGOUS INSECTS. XIII. Kairomonal Activity for *Trichogramma* spp.[1] of Abdominal Tips, Excretion, and a Synthetic Sex Pheromone Blend of *Heliothis zea* (Boddie)[2] Moths[3,4]

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Abstract—Volatile chemicals emanating from an excretion (apparently meconium) and abdominal tips of female *Heliothis zea* (Boddie) moths mediated increased rates of parasitization of *H. zea* eggs by *Trichogramma pretiosum* Riley. A blend of synthetic chemicals, consisting of hexadecanal, (*Z*)-7-hexadecenal, (*Z*)-9-hexadecenal, and (*Z*)-11-hexadecenal, which has been identified as the sex pheromone of and from the abdominal tip of female *H. zea* moths, also increased rates of parasitization of *H. zea* eggs by *T. pretiosum* in greenhouse experiments. In addition, parasitization of *H. zea* eggs by wild *Trichogramma* spp., in field plots of cotton, *Gossypium hirsutum* L., treated with a similar blend of chemicals, in Conrel fibers, was more than double that in untreated plots.

Key Words—Kairomone, pheromone, *Trichogramma, Heliothis zea.*

INTRODUCTION

The involvement of kairomones in the host-seeking behavior of *Trichogamma* spp. has been known for some time. Laing (1937), for example, found that *Trichogramma evanescens* Westwood females perceived an "odor" left at

oviposition sites by adult *Sitotroga cerealella* (Olivier). Lewis et al. (1971) demonstrated that a factor(s) associated with the presence of adult *Heliothis zea* (Boddie) increased rates of parasitization by *T. evanescens*. Lewis et al. (1972) demonstrated that scales from *H. zea* moths were a source of kairomone(s) that elicited increased rates of parasitization and subsequently this interaction has received considerable study (Lewis et al., 1975, 1979; Nordlund et al., 1976; Beevers et al., 1981; Gross et al., 1975). We found that contact with these kairomones stimulated an intensive search behavior and that the kairomone could be used in the field to increase rates of parasitization.

Recently, we demonstrated the involvement of volatile chemicals from plants in the host-seeking behavior of laboratory-reared *Trichogramma pretiosom* and wild *Trichogramma* sp. (Altieri et al., 1981). The finding that *Trichogramma* responded to these plant-produced chemicals, along with other evidence for the existence of long-range mediators, led us to examine *H. zea* moths for active volatile chemicals. In this paper, we discuss the finding that increased rates of parasitization by *Trichogramma* are elicited by volatile materials from *H. zea* abdominal tips and from a "pinkish" excretion of the moths (believed to be meconium since it is present in the gut of pupal stage *H. zea* and absent in the gut of moths soon after emergence) and by blends of synthetic chemicals known to be present in the abdominal tips of female *H. zea* adults and which have been identified as the sex pheromone of *H. zea* (Klun et al., 1980).

METHODS AND MATERIALS

The *Trichogramma* stock used in the greenhouse experiments originated from Hermosilla, Mexico. It was found to cross successfully with a stock from Los Mochis, Mexico (Gonzales and Allen, 1975; Division of Biological Control, University of California, Riverside, California 92501, unpublished results), that was identified as *Trichogramma pretiosum* Riley (Oatman et al., 1970). These parasitoids were reared in our laboratory, according to the procedure of Lewis and Redlinger (1969), in *H. zea* eggs at ca. 26° C and 70% relative humidity, and used on the day of emergence.

The *H. zea* eggs used in the studies (and also for rearing the *T. pretiosum*) were obtained from laboratory cultures, washed with sodium hypochlorite as described by Burton (1969), irradiated with 25 krad (^{60}CO source) when 8–36 hr old, and stored at ca. 10° C.

In the greenhouse experiments, cotyledonous-stage, pink-eyed purple-hull cow peas, grown in 22.8-cm pie pans, were used. Eggs were applied to cotyledons using a camel's hair brush moistened with saliva. Newly emerged *T. pretiosum* were released from 2-dram shell vials.

The synthetic blend used in experiment 3 consisted of (*Z*)-11-hexadecenal, (*Z*)-9-hexadecenal, (*Z*)-7-hexadecenal, and hexadecanal in an 87:3:2:8 ratio (by weight) (Klun et al., 1980, Carpenter et al., 1981). The components used in both the greenhouse and field experiments were obtained from Chemical Samples Company, Columbus, Ohio. For the field experiment, the components were purified by high-performance liquid chromatography on a 25 × 2.5-cm (OD) $AgNO_3$-coated silica column eluted with toluene (Heath and Sonnet, 1980). Each compound collected from the $AgNO_3$ column was filtered through a 10 × 1-cm (ID) column of NaCl and then through a silica gel (60–120 mesh, BDH Chemicals) column of the same dimensions, and solvent was removed by evaporation. The compounds were then analyzed by gas chromatography on a 66-m × 0.25-mm (ID) glass capillary column coated with SP2340 (Supelco, Bellefonte, Pennsylvania) (Teal et al., 1981). This column separates both geometrical and positional isomers of the compounds of interest. The purity of the compounds used in this formulation was greater than 98%. Conrel black hollow fibers (Albany International, Controlled Release Division, Needham Heights, Massachusetts 02194) were evacuated and filled with a blend of compounds consisting of hexadecanal, (*Z*)-7-hexadecenal, (*Z*)-9-hexadecenal, (*Z*)-11-hexadecenal (4%, 1%, 2%, and 92%, respectively, by weight) and 1% BHT [2,6-bis(1,1-dimethylethyl)-4-methyl phenol], an antioxidant, dissolved in an equal volume of *n*-hexane (nanograde, Mallinckrodt) at ca. 208 μg/fiber.

Percentage of parasitization was determined by dissecting the host eggs according to the procedure of Lewis and Redlinger (1969).

Arcsin transformations were conducted on percentages prior to analysis. A paired *t* test was used to determine significance. Means are given $\pm 1\ S\bar{x}$ in parentheses.

RESULTS

Greenhouse Experiments

Experiment 1—Effect of Moth Excretion. This experiment was conducted on individual pans of peas, ca. 1.5 m apart. *H. zea* eggs (10/pan) were applied to the cotyledons in each pan as previously described. Portions (ca. 1 cm^2) of *H. zea* cage liners with a "pinkish" excretion from the moths were clipped from the liners. The liners were obtained from cages of moths less than 36 hr of age. These pieces of liner were placed in the bottom of 2-dram shell vials. Seven vials were then placed, open end up, under the canopy and on the bottom of each treated pan. The control pans received no treatment. Six female *T. pretiosum* were released in each pan and allowed to function for ca. 2 hr. The eggs were then collected and dissected. This experiment was conducted on three different days with 12–20 replications per day, for a total

of 47 replications. The mean percent parasitization in the treated pans was 74.2% (±10.2), which was significantly higher ($P < 0.05$) than the mean percent parasitization of 46.8% (±7.0) for the control pans. Periodic examination indicated that no *Trichogramma* were visiting the vials and thus the *Trichogramma* appear to have detected volatiles emanating from the vials and were stimulated to increase parasitization without direct contact with the chemical source.

Laboratory observations were made of 15 *Trichogramma* females exposed to pieces of the excretion carefully scraped from the liners, so as to contain no other material, and placed on clean filter paper. All the *Trichogramma* individuals became highly excited when they approached the material and began an intensive and fast-paced, weaving search of the substrate. These data demonstrated that there is a chemical(s) in the "pinkish" excretion of adult *H. zea* that enhances search by *T. pretiosum* females and the chemical appears to be volatile.

Experiment 2—Effect of Abdominal Tip Components. In this experiment, pans of peas were set up individually ca. 1.5 m apart. *H. zea* eggs (10/pan) were applied to the cotyledons in each pan, as previously described. *H. zea* female abdominal tips were cut from virgin females and one tip was squashed in a piece of Whatman No. 1 filter paper (ca. 6 cm^2). Two of these papers were placed on the substrate under the canopy of each of the treated pans. The control pans received no such treatment. Six female *T. pretiosum* were released in each pan and allowed to search for ca. 2 hr. Again, the impregnated papers were periodically examined to confirm that no contact stimulation was occurring. The eggs were then collected and dissected, as previously described. This experiment was conducted on six different days with 10 replications per day for a total of 60 replications.

The mean percent parasitization in the treated group was 55.0% (±7.0), which was significantly higher ($P < 0.05$) than the mean for the control group, which was 42.5% (±5.9). These data indicate that some volatile chemical(s) associated with the abdominal tips of *H. zea* moths increased rates of parasitization by *T. pretiosum*.

Experiment 3—Use of Synthetic Pheromone Blend. In this experiment, pans of peas were arranged in groups of three (close enough together that the foliage touched) with ca. 0.75 m between groups on greenhouse tables. *H. zea* eggs (10/pan) were placed on the cotyledons in each pan as previously described. The synthetic pheromone blend was applied to two cotton rolls (#2 Medium, Uni-Disco Inc., P.O. Box 4450, Detroit, Michigan 48228) at the rate of 5.0 μg/roll in 0.5 ml of hexane. Two rolls were placed on the table in the center of each group of treated pans. The control groups received no such treatment. Two vials of 6 female *T. pretiosum* each were released under the foliage, on opposite sides of each group. The parasitoids were allowed to

search for ca. 3 hr. The eggs were then collected and dissected. This test was conducted on 3 days with 6 replications each day, for a total of 18 replications.

The mean rate of parasitization in the treated groups was 52.8% (±12.5), which was significantly higher ($P < 0.05$) than the mean of 40.8% (±10.1) for the control groups. These data demonstrated that the sex pheromone blend of *H. zea* can increase parasitization by *T. pretiosum.*

Field Experiment Using Synthetic Pheromone

Pairs of plots, 1 row by 1.5 m, were set up in a field of cotton, *Gossypium hirsutum* L. (variety: Delthine 61), that was ca. 0.75 m high. Plots were separated from each other by a minimum of 15 m. In the treated plots, Conrel fibers loaded with the pheromone blend, as previously described, were placed 3 each on a piece of Scotch Doublestick Tape (Minnesota Mining and Manufacturing Co., St. Paul, Minnesota 55101) and attached to a stem in the upper third of the plant at four equally spaced locations (total 12 fibers/plot). The control plots were untreated. All plots were egged with 20 *H. zea* eggs, using a camel's hair brush and Plantgard as an adhesive (Nordlund et al., 1974). After ca. 4 hr exposure, the eggs were collected and dissected as described. The test was run for 2 days with one reading each day. No parasitoids were released, thus the parasitization that occurred resulted from naturally occurring *Trichogramma.* This experiment was replicated 10 times on two different occasions (at different locations), for a total of 20 replications.

The results are given in Table 1 and demonstrate that the presence of the sex pheromone blend of *H. zea* elicits a dramatic increase in the rate of parasitization by naturally occurring *Trichogramma* in the field. The parasitization in the sex-pheromone-treated plots was more than double that

TABLE 1. MEAN PERCENTAGE PARASITIZATION (±1$\bar{x}$) OF *H. zea* EGGS BY NATURALLY OCCURRING *Trichogramma*, IN FIELD PLOTS TREATED WITH CONREL FIBERS LOADED WITH SYNTHETIC *H. zea* SEX PHEROMONE[a]

	Treated	Control
Reading 1 (day 1)[b]	24.4 (±5.9)	18.6 (±4.8)
Reading 2 (day 2)[c]	45.5 (±10.2)	20.2 (±5.0)
Means of both readings[d]	35.6 (±8.1)	20.6 (±4.9)

[a]Data from 20 replications.
[b]Means not significantly different ($P < 0.05$).
[c]Means significantly different ($P < 0.01$).
[d]Means significantly different ($P < 0.001$).

of the control plots during the second day. The significantly higher parasitization in the treated plots on the second day of the test as compared to the slight difference on the first day indicates that the increase was a result of the attraction and resulting redistribution of the parasitoids rather than simply stimulation of parasitoids that were already present.

DISCUSSION

Previous studies reported earlier in this series and elsewhere have demonstrated that the contact stimuli in the scales of *H. zea* moths serve a vital role in the host-finding behavior of *Trichogramma* spp. and that these chemicals can be used to manipulate the behavior and increase the field performance of *Trichogramma* (Lewis et al., 1975, 1979). However, questions remained as to whether these stimuli were, in fact, the only stimuli to which *Trichogramma* respond. Earlier preliminary screening, using various olfactometers, for attraction of *Trichogramma* to long-range volatile chemicals resulted in no positive results. However, evidence for the existence of such mediators with a very strong influence on the foraging behavior of *Trichogramma* continued to mount. Field releases of *H. zea* moths (wings partially clipped to prevent migration from the field) into a 5-acre cotton field at Tifton, Georgia, resulted in a dramatic increase of natural *Trichogramma* within 2–3 days (Morrison and Lewis, unpublished data) indicating the keen ability of *Trichogramma* to detect the presence or activity of *H. zea* moths. Studies with biweekly releases of ca. 50,000 *Trichogramma* in a large scale study in Portland, Arkansas, during the 1981 growing season, reflected increased parasitization as the moth population increased but a rapid drop in rates of parasitization at the end of and between generations, indicating a keen ability of the *Trichogramma* to perceive and quickly respond to changes in moth populations (King et al., unpublished data). Similar *Heliothis–Trichogramma* relations were noted by Lingren (personal communications) in various *Trichogramma* studies in cotton-growing areas of Texas. The data reported here demonstrate the ability of *Trichogramma* to utilize the sex pheromone and other volatile chemicals from *H. zea* moths as kairomones for quick and effective detection of habitats containing host eggs and offer an explanation for the responses cited above.

Such a relationship is of ecological significance in that it provides a mechanism for *Trichogramma* to synchronize with the initial upsurge of moth populations and maximum egg deposition, thereby avoiding the competition with predators that tends to build up later in a generation. Host eggs freshly parasitized by *Trichogramma* are readily consumed by egg predators. However, after the host egg has been parasitized for 3–4 days, it becomes less desirable for the predators (unpublished data).

The identity of the chemical(s) in the "pinkish" excretion that elicits the

response by *Trichogramma* is unknown and needs immediate further attention. The majority of the tissue, obtained when cutting the abdominal tips, was the epidermal gland from which Klun et al. (1980) identified the sex pheromone components used in experiment 3 and the field experiment. Additional studies will be required to determine if other active materials are present in the gland.

The fact that various pheromones or components of pheromones also serve as kairomones for predators or parasitoids has been demonstrated on several occasions. Rice (1969), for example, found that the predators *Enoclerus lecontei* (Wolc.) and *Temnochila virescens chlorodia* (Mann.) and the parasitoid *Tomicobia tibialis* Ashm. respond to the aggregation pheromone of their host or prey, *Ips* spp. Vité and Williamson (1970), found that frontalin, the major component of the aggregation pheromone of *Dendroctonus frontalis* Zimm. also attracts the predator *Thanasimus dubius* (F.). *Aphytis melinus* DeBach and *Aphytis coheni* DeBack, two parasitoids of *Aonidiella aurantii* (Mask.), are attracted to the sex pheromone of *A. Aurantii* (Sternlicht, 1973). Kennedy (1979) found that *Cheriopchus colon* (L.), *Entedon leucogramma* (Ratz.), and *Dendrosoter protuberans* Nees, parasitoids of *Scolytus multistriatus* (Marsham) and *Cerocephala rufa* (Walker), a reported hyperparasitoid of *D. protuberans*, are attracted to multilure, the sex pheromone of *S. multistriatus*. Male *Nazara viridula* (L.) produce a sex pheromone that is highly attractive to females of the same species and to the tachinid parasitoid *Trichopoda pennepes* (F.) (Mitchell and Mau, 1971). Also, Corbet (1971) found that the mandibular gland secretion of *Anagasta kuehniella* (Zeller) functions as an epideictic pheromone and elicits ovipositor probing by the parasitoid *Venturia canescens* (Grav.).

The use of volatiles, as demonstrated in these studies, to enhance the field performance of *Trichogramma*, should require considerably less concern with application patterns than is the case with contact stimuli (Lewis et al., 1979; Beevers et al., 1981). The technology developed for use of sex pheromones for mating disruption should contribute readily to their application for enhancing the performance of *Trichogramma*.

These new discoveries of the overlapping roles of chemical cues in the mating behavior of *H. zea* and host-search behavior of *Trichogramma* open exciting possibilities for integration of augmentation–manipulation of entomophages and mating disruption into a potentially powerful pest management system for *H. zea* and similar pests.

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